Recent changes in the population of *Stagonosporopsis tanaceti*, the cause of ray blight of pyrethrum in Australia

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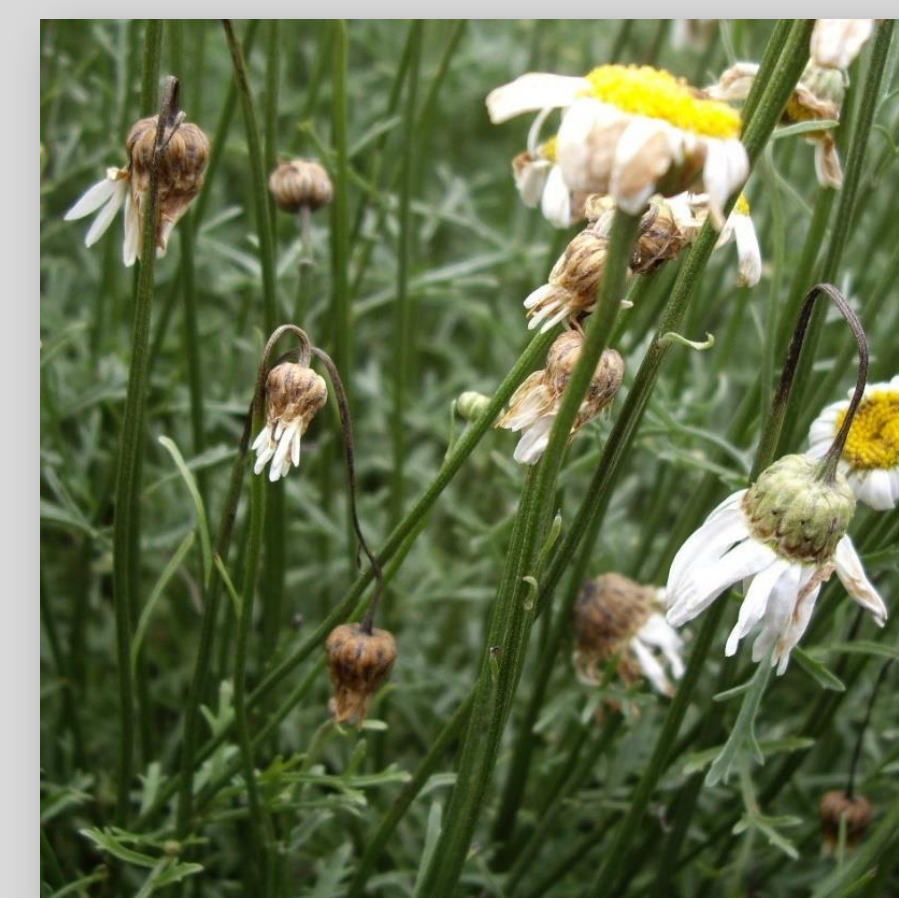THE UNIVERSITY OF MELBOURNE

Botanical Resources AUSTRALIA

Pyrethrum is a perennial species in the *Asteraceae*, and the source of natural pyrethrins. Australia has the largest pyrethrum industry worldwide, supplying ca. 70% of the global pyrethrin demand.

Ray blight, caused by *Stagonosporopsis* spp., occurs on various *Asteraceae* worldwide, causing leaf lesion and flower blight. Ray blight of pyrethrum in Australia is caused by *S. tanaceti*.

Disease control depends on heavy use of fungicides; no resistant pyrethrum varieties are yet available.

Tanacetum cinerariifolium   Ray blight symptoms

Little is known about the population structure and evolutionary potential of *S. tanaceti* in Australia.

The aim was to develop multiplexed microsatellite markers for *S. tanaceti* and apply these to:

- Investigate the possible geographical   structure in *S. tanaceti* populations

- Identify any temporal changes in the pathogen's genetic diversity and composition

- Test for sexual reproduction of the pathogen in the field in Australia

Microsatellite library development

A partial *de novo* genome of *S. tanaceti* was assembled and used for microsatellite discovery. Of the 44 primer pairs tested, 13 gave reproducible polymorphic bands in *S. tanaceti*. Also, 12 (27%) and 20 (22%) markers were transferrable to *S. inoxydabilis* and *S. chrysanthemi*, respectively.
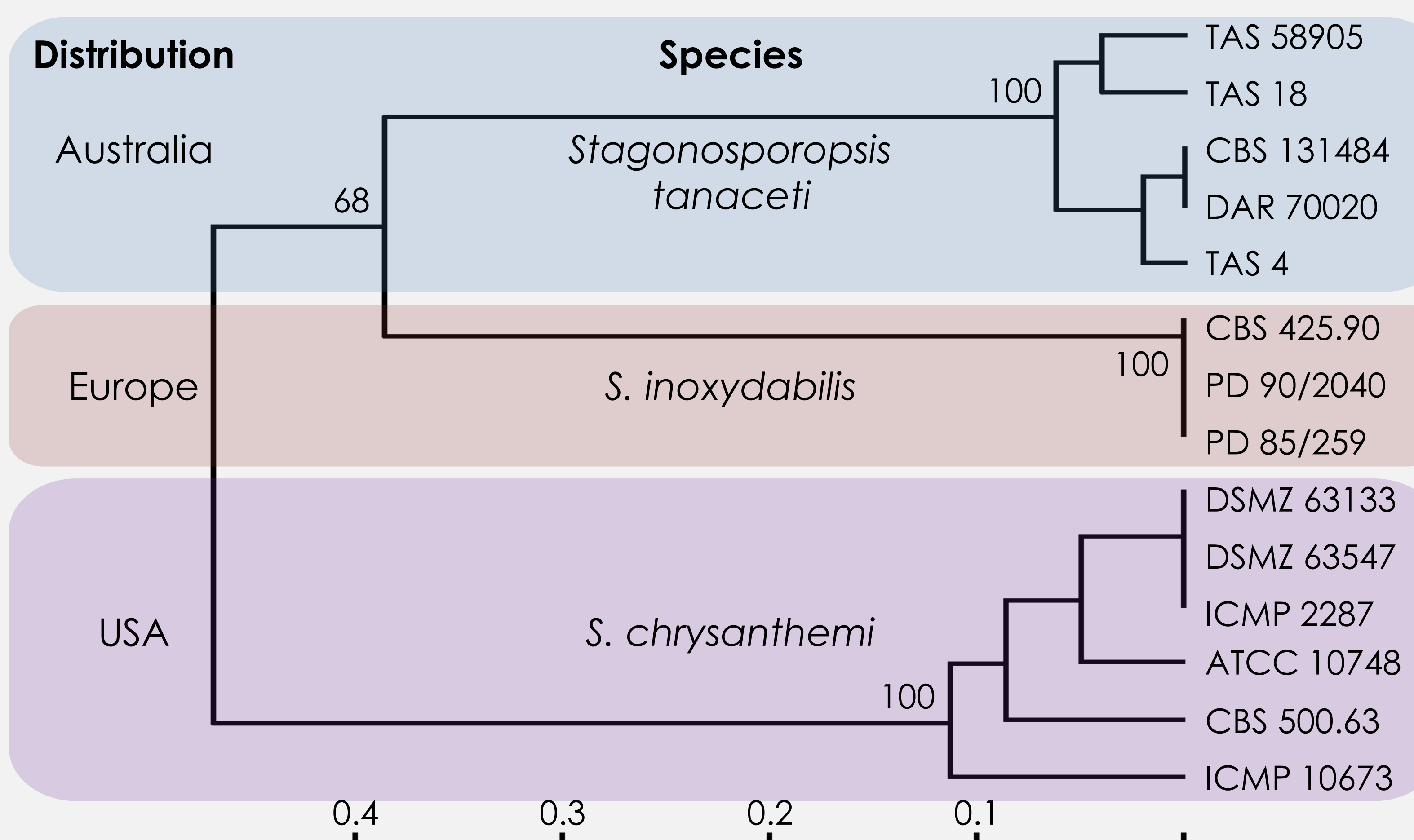

Fig1. UPGMA tree based on Bruvo's distance using seven microsatellite markers that were transferrable across species

Conclusions

• Linkage disequilibrium, high clonality and lack of recombinant haplotypes confirmed the absence/very low levels of sexual reproduction

• Two hypotheses are proposed to explain the major change in the genetic composition of *S. tanaceti* over time:

Hypothesis 1: Detection of previously unobserved clonal lineages in 2012 (pink sub-network in Fig 2) may be the result of a second introduction of *S. tanaceti* to the pyrethrum growing region

Hypothesis 2: Rapid increase in the frequency of the newly discovered haplotypes in 2013 suggests presence of strong selection

• The rapid change in population composition coincided with a switch of fungicides and release of new pyrethrum varieties. Future studies need to investigate the pathogenicity and fungicide resistance of the newly detected haplotypes

References

Vaghefi *et al*. 2012. *Stagonosporopsis* spp. associated with ray blight disease of *Asteraceae*, *Australasian Plant Pathology* 41: 675–686

Vaghefi *et al*. 2014. Identification of the *MAT1* locus in *Stagonosporopsis tanaceti*, and exploring its potential for sexual reproduction in Australian pyrethrum fields, *Fungal Biology*, http://dx.doi.org/10.1016/j.funbio.2014.04.004
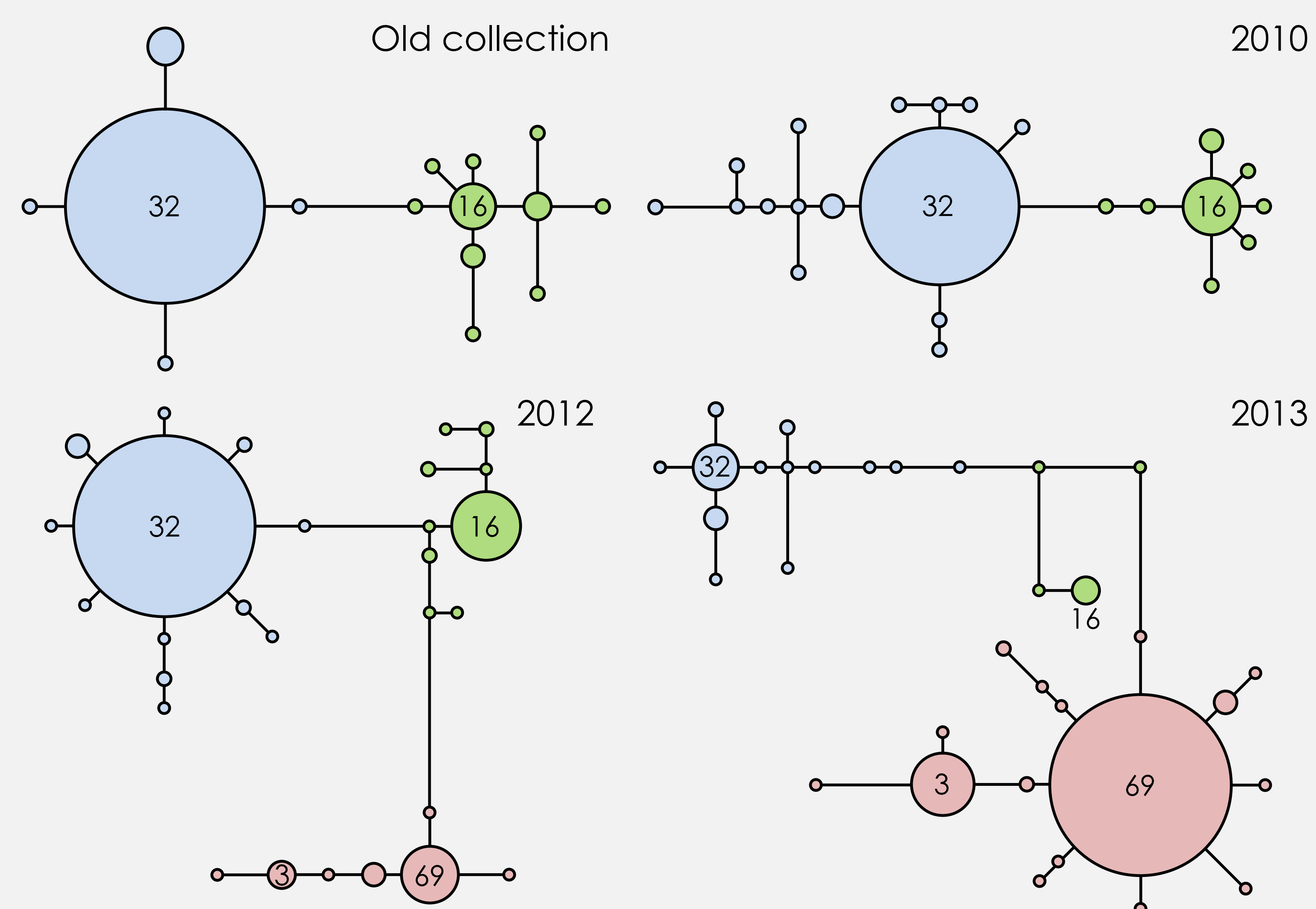
Population analyses

In total, 407 *S. tanaceti* isolates were genotyped. This included isolates from 2010, 2012 & 2013 as well as an old population collected from 1998 to 2004. AMOVA results showed a higher proportion of genetic variation existed among years (42 %) than among localities within years (9.5 %).

Diversity indices	Population			
	Old collection	2010	2012	2013
Sample size (N)	46	40	178	143
Allelic richness after rarefaction (R_α)	2.4	2.8	2.7	2.7
Gene diversity (H_e)	0.26	0.27	0.41	0.35
No. of Multi-locus haplotypes (MLHs)	15	23	28	33
Expected no. of MLHs	14	23	14	15
Clonal fraction	0.67	0.43	0.84	0.77
Evenness (ED)	0.51	0.54	0.74	0.69
Genotypic diversity (D*)	0.75	0.88	0.79	0.79
Vo/Ve ($P(Vr>=Vo)$)	3.24 (0.001)	2.64 (0.001)	4.34 (0.001)	13.5 (0.001)
$\bar{r}_d$ (P value)	0.18 (0.001)	0.09 (0.001)	0.22 (0.001)	0.31 (0.001)

Fig 2. Minimum spanning networks constructed based on Bruvo's distance in the R package *Poppr*. Each circle represents a multi-locus haplotype (MLH), with the size proportional to the MLH frequency. Edge lengths are proportional to the genetic distance between the MLHs. Colours indicate the clusters determined by the Discriminant Analysis of Principal Coordinates (DAPC) in the R package *Adegenet*.